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(54) Stabilized Polysaccharides

(57) The invention relates to the stabilization of meningococcal polysaccharides and provides a composition which comprises:

(a) one or more meningoccocal polysaccharides, and

(b) a compound which is capable both of forming a hydrate and of reversible dehydration under the conditions of lyophilization, or a mixture of two or more such compounds, with the exception of lactose alone.

Typical of such compounds are saccharose, raffinose, glucose, tochalose, glycerophosphate and glutaminate.

The composition may be an intermediate product in the production of a vaccine against meningitis or may be in the form of a meningitis vaccine. The composition is preferably lyophilised.

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SPECIFICATION

Stabilized Polysaccharides

The invention relates to the stabilization of meningococcal polysaccharides, in particular, to the stabilization of meningococci capsule polysaccharides of group A. The invention further relates to the preparation of a vaccine against meningococcal diseases, which vaccine comprises stabilized meningococcal polysaccharides.

Vaccines against meningitis caused by meningococci contain as the essential active component group-specific polysaccharides which can be isolated from meningococci of the serological groups A and C. The polysaccharides are isolated from cultures of meningococci and are purified by various precipitation and extraction measures (cf. E. C. Gotschlich *et al.*, Progr. Immunbiol. Standard., Vol. 5, pp. 485—491, Karger, Basel, 1972 and WHO Technical Report Ser. No. 594, pages 66/67, 1976).

Meningococcal polysaccharides and the vaccines prepared therefrom often have unsatisfactory stability on storage, which affects the immunizing effect of the active substances. Even in the dry, for example, lyophilized state, meningococci vaccines show an extraordinary instability: even with a water content of less than 1% in a dry mass, the polysaccharide material hydrolyses to a detectable degree within a few months when stored at about 4°C, and within a week at higher temperatures. The hydrolysis of meningococcal polysaccharides leads to a depolymerization and therewith to an undesirable reduction of the immunogenicity of a vaccine which contains them. (The depolymerization can be followed by determining the change in the molecular weight using, for example, chromatographic techniques.) Meningococcal polysaccharides and meningococci vaccines are therefore generally stored in a deep-frozen state.

It has been proposed as a result of investigations made by the Rijks Institut voor the Volkgezondheid, Bilthoven, (Bull. Wdl. Hlth. Org., *55* 43—98, 1977), that lactose considerably improves the stability on storage of meningococcal polysaccharides. An explanation of the increase in stability afforded by lactose over that afforded by the hitherto used but unsatisfactory mannitol could not be given.

The present invention is based on the surprising observation that hydrate-forming compounds which are dehydrated under the conditions of lyophilization and subsequently hydrate reversibly are capable of stabilizing meningococcal polysaccharides.

The present invention accordingly provides a composition which comprises

(a) one or more meningococcal polysaccharides and

(b) a compound which is capable both of forming a hydrate and of reversible dehydration under the conditions of lyophilization, or a mixture of two or more such compounds, with the exception of lactose alone.

Although the stabilizing compounds, that is to say, the compounds which are capable both of forming a hydrate and of reversible dehydration under the conditions of lyophilization, individually impart to the meningococcal polysaccharides a good stability after lyophilization of a solution or suspension thereof, it may be preferable to use the stabilizing compounds not alone but together with other compounds to stabilize meningococcal polysaccharides, for example, compounds previously proposed for stabilizing meningococcal polysaccharides, and substances facilitating lyophilization, for example, so-called cake-formers, for example, glycine and mannite.

A mixture of stabilizing compounds according to the invention may show a synergistic effect and further improve the stability to storage of the meningococcal polysaccharides. Such synergism is observed in particular in the case of mixtures of two of the stabilizing compounds in a molar ratio of from 1:2 to 2:1.

The preferred stabilizing compounds are saccharose, raffinose, glucose, trehalose, glycerophosphate and glutaminate. Saccharose and raffinose are especially preferred, either alone or together with each other and/or with one or more of the other above-mentioned stabilizing compounds. Any one or more of the stabilizing compounds may be used together with lactose.

Preferably at least 0.3 mg and advantageously from 1 to 100 mg of a stabilizing compound or a mixture of two or more thereof is used per 100 μg of meningococcal polysaccharides: when the composition of the invention is in liquid form, generally as an aqueous preparation, the above-mentioned amounts of the various components are preferably present per ml.

The composition of the invention may be lyophilized. This is preferable if it is to be stored.

The composition of the invention may be an intermediate product in the manufacture of a vaccine, or may itself be in the form of a vaccine, for example, a vaccine suitable for direct parenteral administration or a lyophilized vaccine to which a suitable liquid, for example, pyrogen-free water or a physiological sodium chloride solution is added before administration.

A vaccine comprising one or more meningococcal polysaccharides and one or more stabilizing compounds (with the exception of lactose alone), is accordingly a preferred embodiment of the invention.

It will be appreciated that if a composition of the invention is a vaccine, the content of stabilizing compound(s) should not be so high that the vaccine for administration is hypertonic, for example, as occurs with saccharose when present in an amount over 100 mg/ml. Preferably at least one of the stabilizing compounds is used in a concentration of from 5 to 20 mg/ml of vaccine to be administered. (A vaccine to be administered is the vaccine in suitable liquid form, either as such or after reconstitution from a

lyophilized vaccine using a suitable liquid.)

For preparing the meningococcal polysaccharides it is preferable to proceed according to WHO Technical Report Ser. No. 594, pages 66/67, 1976, as follows:

A meningococci culture or the supernatant obtained from a culture of meningococci is combined with 0.1 to 0.3% of cetyltrimethylammonium bromide (Cetavlon) and the resulting precipitate is isolated after 2—24 hours by sedimentation or centrifugation. The sediment is subsequently extracted with a 0.8—2 molar, preferably 1 molar, aqueous solution of calcium chloride, combined with up to 20 to 30%, preferably 25% (v/v) of ethanol and the supernatant is then isolated by centrifugation. An intermediate product is precipitated from the supernatant by the further addition of up to 70—85%, preferably 80% (v/v) of ethanol, and is isolated by centrifugation.

The intermediate product is then taken up in a slightly acid or neutral to weakly basic aqueous medium, for example, having a pH from 6 to 9, for example of 0.1—0.4 M sodium acetate solution, and this preparation is then extracted with phenol. Generally, a buffer-saturated phenol solution is used (composition, for example, 100 g of phenol+40 ml of buffer). The resulting aqueous phase is dialyzed against a 0.1 M calcium chloride solution and then subject to ultracentrifugation. The polysaccharide is then precipitated with the aid of an alcohol, preferably ethanol, and dried. The material may be stored at —20°C if it is not required immediately.

At least one stabilizing compound, optionally with one or more additives, is incorporated, in accordance with the invention, with the maningococcal polysaccharide obtained as described above and the mixture is lyophilized.

The invention also provides an improvement in the method of stabilization of meningococcal polysaccharide(s) by the incorporation of lactose, which comprises incorporating with an aqueous solution of the meningococci polysaccharide(s) instead of the total amount of lactose or a part thereof, at least 0.3 mg, per 100 μg of polysaccharide(s), of at least one compound which is capable of reversible dehydration under conditions of lyophilization and which forms a hydrate, and drying the mixture obtained.

The following Examples illustrate the invention.

Example 1

Meningococcal polysaccharides were prepared according to the procedure of the WHO Technical Report Ser. No. 594, pages 66/67. 1976 and were brought into the form of a lyophilized vaccine having the following composition.

	mg
N. meningitidis group A polysaccharide	2.5
Saccharose	250

During storage for 3 months at 37°C, the lyophilized product showed practically no decrease in the degree of polymerization. The Kd-value, which was 0.26 at the beginning of storage, rose to 0.29. In contradistinction thereto, a sample stabilized with lactose under the same conditions showed an increase in the Kd-value from 0.26 to 0.35.

For administration as a vaccine, the lyophilisate is dissolved in 25 ml of a physiologically tolerable solvent, preferably a solution which contains 7 mg of NaCl/ml; 0.015 mol/l of sodium phosphate buffer, pH 7.4, and 0.05 mg of sodium timerfonate/ml.

Example 2

A vaccine was prepared according to Example 1 but having the following composition:

	mg
N. meningitidis group A polysaccharide	2.5
Lactose	125
Saccharose	125

During storage of the lyophilized material at 56°C, the Kd-value of the polysaccharide rose within 2 months from 0.26 to 0.48. Products combined with 250 mg of lactose of 250 mg of saccharose showed after the same period of time Kd-values of >0.7.

K_d is determined by chromatography on Sepharose 4B ("Sepharose" is a Trade Mark) and is defined by the following equation:

$$K_d = \frac{V_e - V_o}{V_T V_o}$$

V_e=The volume of the eluate from the start of the chromatography to the elution maximum of the main component of the polysaccharide antigen.

V_o=The elution volume of a totally excluded substance, for example, high molecular Blue Dextran.

V_T=The elution volume of a totally included substance, for example, radio-actively labelled water.

This definition means that $0 \leqslant K_d \leqslant 1$. The limit values are reached with a completely excluded substance ($K_d=0$) or with a completely included substance ($K_d=1$). With a higher Kd-value, the size of the molecule is reduced. In the present case, a rising K_d-value indicates that the meningococci-polysaccharide depolymerizes.

Claims

1. A composition which comprises
(a) one or more meningococcal polysaccharides and
(b) a compound which is capable both of forming a hydrate and of reversible dehydration under the conditions of lyophilization, or a mixture of two or more such compounds, with the exception of lactose alone.

2. A composition as claimed in claim 1, wherein component (b) is saccharose and/or raffinose.

3. A composition as claimed in Claim 1, wherein component (b) is glucose, trehalose, glycerophosphate, or glutaminate.

4. A composition as claimed in Claim 1, wherein component (b) comprises two or more compounds selected from saccharose, raffinose, glucose, trehalose, glycerophosphate, glutaminate and lactose.

5. A composition as claimed in Claim 4, wherein component (b) comprises two compounds selected from those specified in Claim 4, the compounds being present in a molar ratio of from 1:2 to 2:1.

6. A composition as claimed in Claim 4, wherein component (b) comprises saccharose and/or raffinose and one or more compounds selected from glucose, trehalose, glycerophosphate, glutaminate and lactose.

7. A composition as claimed in Claim 6, wherein component (b) comprises

(i) succharose or raffinose and

(ii) glucose, trehalose, glycerophosphate, glutaminate and lactose,

the molar ratio of (i) to (ii) being from 1:2 to 2:1.

8. A composition as claimed in any one of Claims 1 to 7, which comprises at least 0.3 mg of component (b) per 100 μg of total meningococcal polysaccharides.

9. A composition as claimed in Claim 8, which comprises from 1 to 100 mg of component (b) per 100 μg of total meningococcal polysaccharides.

10. A composition as claimed in any one of Claims 1 to 9, wherein the meningococcal polysaccharides are obtained from meningococci of group A.

11. A composition as claimed in any one of Claims 1 to 10, in the form of an aqueous preparation.

12. A composition as claimed in any one of Claims 1 to 11, in lyophilized form.

13. A composition as claimed in Claim 1, substantially as described in Example 1 or Example 2 herein.

14. A composition as claimed in any one of Claims 1 to 13, in the form of a vaccine.

15. A method of stabilizing meningococcal polysaccharides, which comprises incorporating with the meningococcal polysaccharides a compound which is capable both of forming a hydrate and of reversible dehydration under the conditions of lyophilization, or a mixture of two or more such compounds, with the exception of lactose alone.

16. A method as claimed in Claim 15, wherein the compound or compounds incorporated with the meningococcal polysaccharides is or are as defined in any one of Claims 2 to 9.

17. Meningococcal polysaccharides whenever stabilized by a process as claimed in Claim 14 or Claim 15.

18. A vaccine which comprises stabilized meningococcal polysaccharides as claimed in Claim 15.

19. An improvement in the method of stabilization of meningococcal polysaccharide(s) by the incorporation of lactose, which comprises incorporating with an aqqeous solution of the meningococci polysaccharide(s) instead of the total amount of lactose or a part thereof, at least 0.3 mg, per 100 μg of polysaccharide(s), of at least one compound which is capable of reversible dehydration under conditions of lyophilization and which forms a hydrate, and drying the mixture obtained.

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